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Supporting information for article:

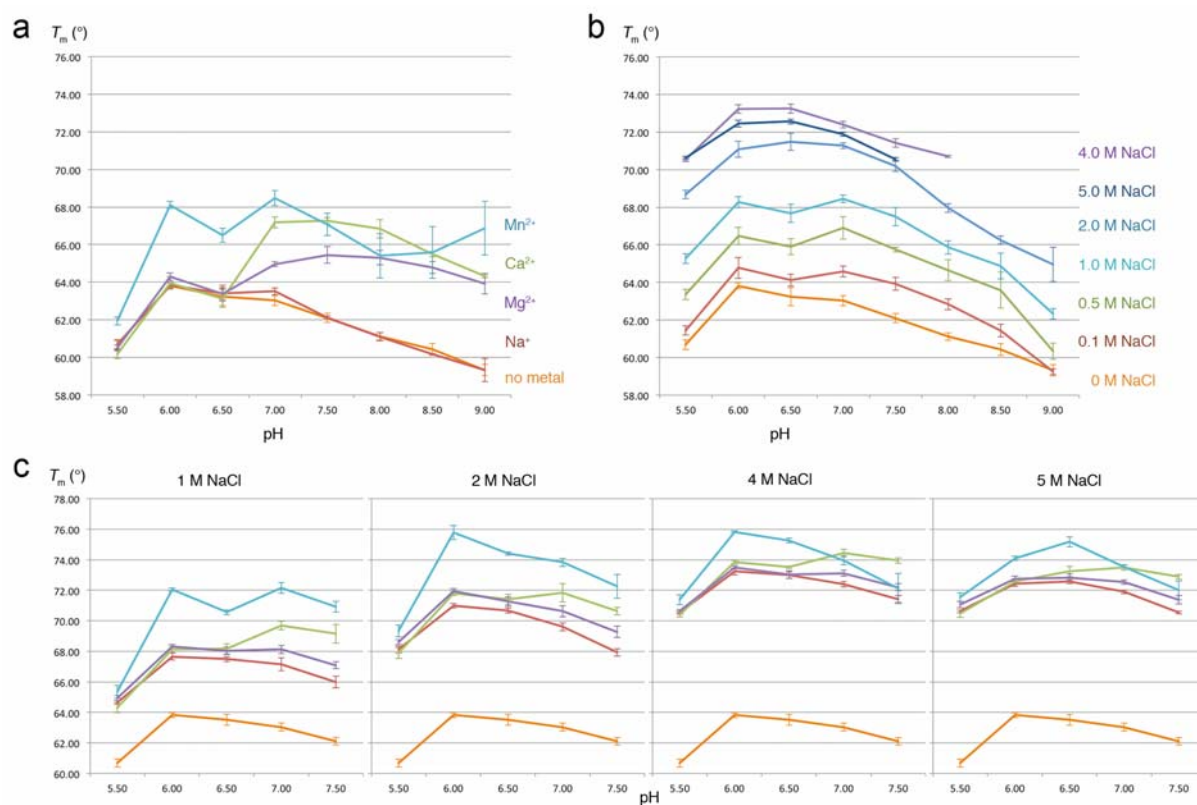
**High-resolution crystal structure of a polyextreme GH43
glycosidase from *Halothermothrix orenii* with α -l-
arabinofuranosidase activity**

**Noor Hassan, Lokesh D. Kori, Rosaria Gandini, Bharat K. C. Patel, Christina
Divne and Tien Chye Tan**

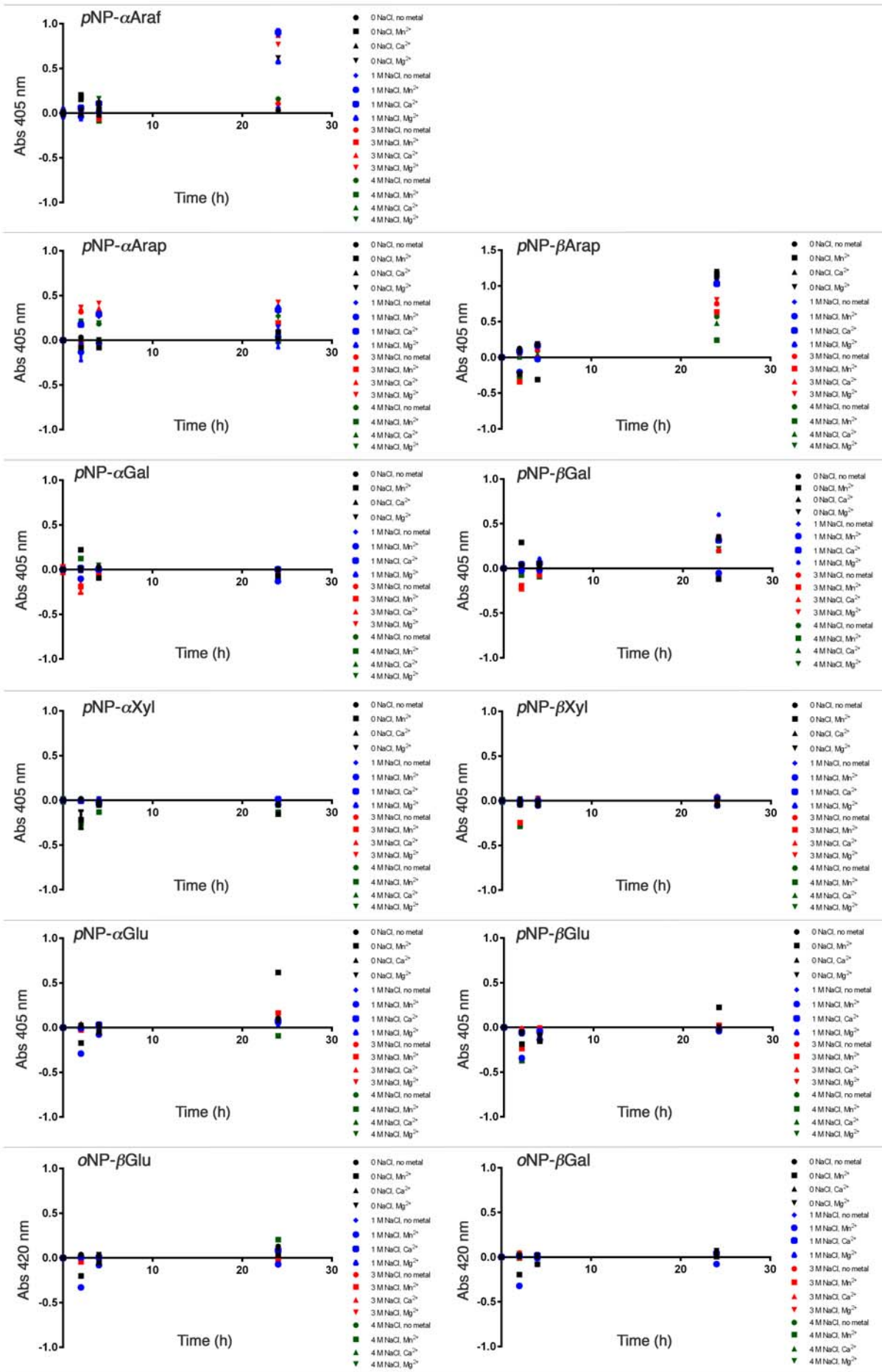
Supplimentary information

High-resolution crystal structure of a polyextreme GH43 glycosidase from *Halothermothrix orenii* with α -L-arabinofuranosidase activity

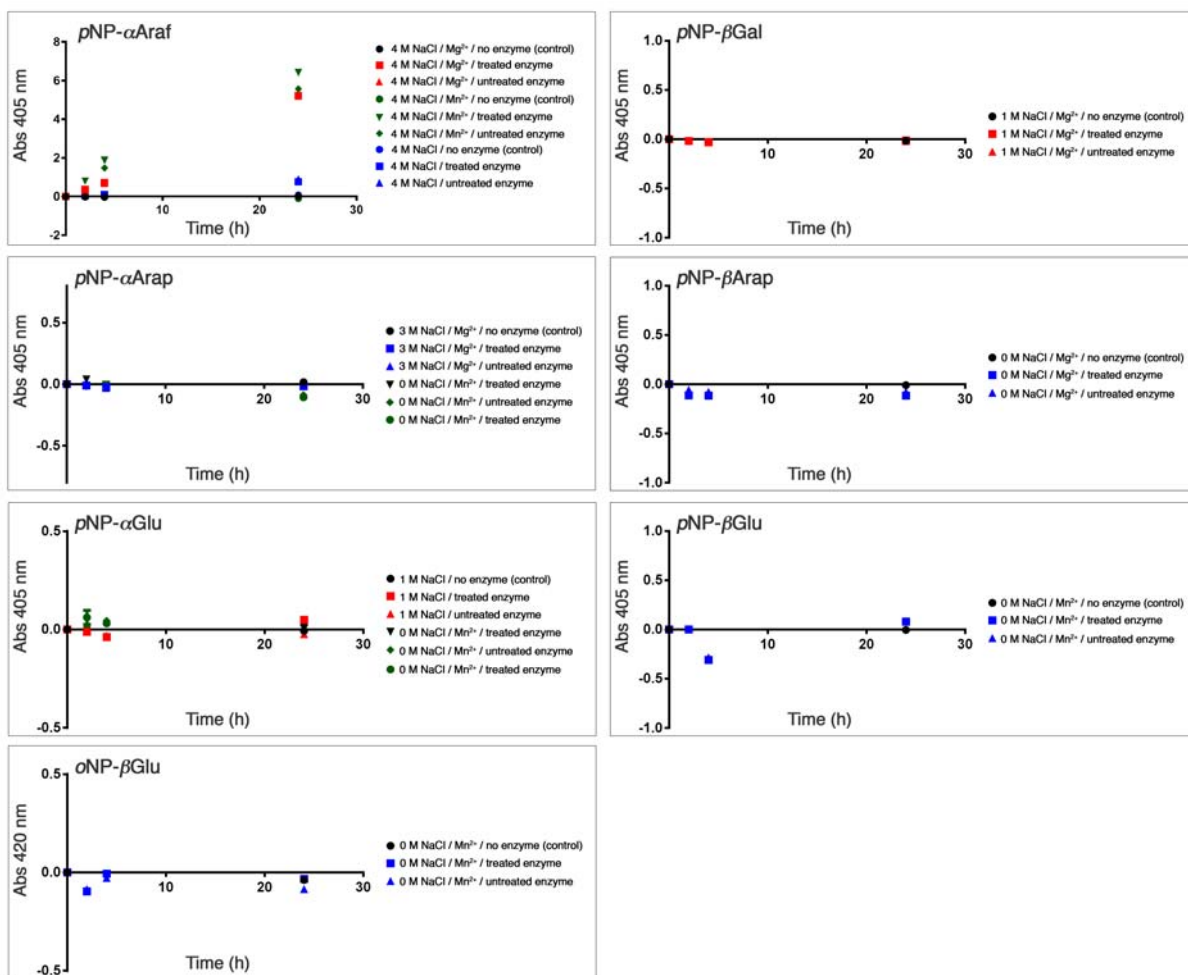
Noor Hassan,^{a,b} Lokesh D. Kori,^{c,d} Rosaria Gandini,^{a,b} Bharat K. C. Patel,^c Christina Divne,^{a,b} and Tien Chye Tan^{a,b*}



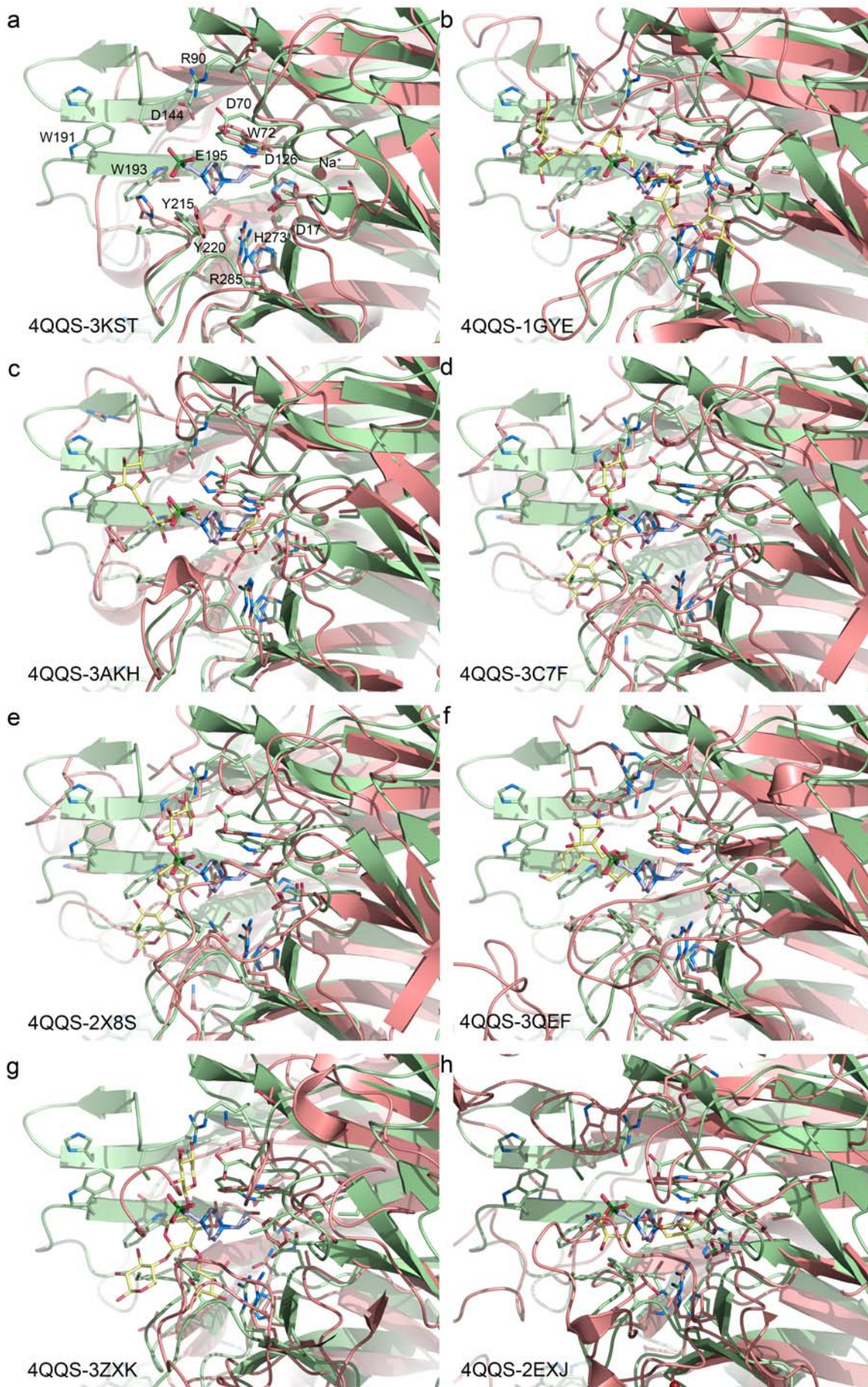
Supplementary Figure S1. Melting temperature of unfolding (T_m) as a function of (a) 2 mM metal ion (Mn²⁺, Ca²⁺, Mg²⁺ and Na⁺), (b) NaCl concentration, and (c) the combined effect of NaCl concentration and metal ion (orange, no metal; red, Na⁺; purple, Mg²⁺; green, Ca²⁺; blue, Mn²⁺).



Supplementary Figure S2. Activity screen using chromophoric substrates. The initial screen was performed using eleven *p*- and *o*-nitrophenyl sugars, various NaCl concentrations (0, 1, 2 and 4 M NaCl), and different divalent cations (2 mM Mn²⁺, Mg²⁺ or Ca²⁺). The release of chromophore was measured as the absorbance at 405 nm for *p*-nitrophenyl and at 420 nm for *o*-nitrophenyl.



Supplementary Figure S3. Activity assay using a subset of substrates and conditions. Assays were performed with both EDTA/EGTA-treated and untreated protein.



Supplementary Figure S4. Comparison of *HoAraf43* (green ribbon) with representative GH43 enzyme-carbohydrate complexes (red ribbon): (a) *B. thetaiotaomicron* endo-1,4- β -xylanase (3KST; r.m.s.d. 1.22 Å for 257 C α atoms); (b) exo- α -1,5-L-arabinanase *CjArb43A* in complex with arabinohexaose (1GYE; Nurizzo *et al.*, 2002; r.m.s.d. 1.63 Å for 230 C α atoms); (c) exo- α -1,5-L-arabinofuranosidase *SaAraf43A* in complex with α -L-arabinofuranotriose (3AKH; Fujimoto *et al.*, 2010; r.m.s.d. 1.62 Å for 251 C α atoms); (d) *B. subtilis* arabinoxylan α -1,3-L-arabinofuranohydrolase in complex with xylotriose (3C7F; Vandermarliere *et al.*, 2009; r.m.s.d. 1.88 Å for 229 C α atoms); (e) *B. subtilis* endo- α -1,5-L-arabinanase *Abn2* in complex with arabinotriose (2X8S; de Sanctis *et al.*, 2010; r.m.s.d. 1.71 Å for 219 C α atoms); (f) *C. japonicus* α -1,2-L-arabinofuranosidase *Abf43A* in complex with α -1,3-arabinofuranose-substituted arabinofuranoside-substituted α -1,5-L-arabinotriose (3QEF; Cartmell *et al.*, 2011; r.m.s.d. 1.70 Å for 231 C α atoms); (g) *H. insolens* *HiAXHd3* in complex with xylotriose (3ZXK; McKee *et al.*, 2012; r.m.s.d. 2.18 Å for 242 C α atoms); (h) *G. stearothermophilus* β -xylosidase *XynB3* in complex with xylobiose (2EXJ; Br \ddot{u} x *et al.*, 2006; r.m.s.d. 1.74 Å for 268 C α atoms). Selected amino acids in *HoAraf43* have been added to panel (a).